Published in final edited form as:

J Org Chem. 2013 November 15; 78(22): 11294–11301. doi:10.1021/jo401846m.

Two Rare-Class Tricyclic Diterpenes with Anti-tubercular Activity from the Caribbean Sponge *Svenzea flava*. Application of Vibrational Circular Dichroism Spectroscopy for Determining Absolute Configuration

Edward Avilés, Abimael D. Rodríguez, and Jan Vicente

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, San Juan, Puerto Rico 00931-3346 United States

Abimael D. Rodríguez: abimael.rodriguez1@upr.edu

Abstract

Two new natural products, **3** and **4**, and their predecessor 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**), of the rare isoneoamphilectane-class of marine diterpenes, along with the known amphilectane diterpenes **6–8**, were isolated from the *n*-hexane extract of the marine sponge *Svenzea flava* collected at Great Inagua Island, Bahamas. The molecular structures of compounds **3** and **4** were established by spectroscopic (1D/2D NMR, IR, UV, HRMS) methods and confirmed by a series of chemical correlation studies. In a first ever case study of the assignment of the absolute configuration of a molecule based on the isoneoamphilectane carbon skeleton, the absolute configuration of compound **5** was established as 3*S*, 4*R* 7*S*, 8*S*, 11*R*, 12*S* and 13*R* by application of vibrational circular dichroism (VCD). *In vitro* anti-TB screenings revealed that metabolites **2–4** and in particular, semi-synthetic analog **5**, are strong growth inhibitors of *Mycobacterium tuberculosis* H₃₇Rv.

Keywords

Isoneoamphilectane; *Svenzea flava*; Marine sponge; Tuberculosis; Vibrational circular dichroism; Diterpene

INTRODUCTION

Diterpenes based on the amphilectane ring skeleton and its variants (Scheme 1), possessing isocyanide, isocyanate, isothiocyanate, and formamide functionalities, have been isolated from marine sponges belonging to the genera *Hymeniacidon*,¹ *Cribochalina*,² *Stylissa*,³ *Cymbastela*,⁴ *Amphimedon* (*Adocia*),⁵ *Halichondria*,⁶ *Axinella*,⁷ and *Pseudoaxinella*.⁸ Among these polycyclic compounds, those that are based on the neoamphilectane and

Correspondence to: Abimael D. Rodríguez, abimael.rodriguez1@upr.edu.

Supporting Information

Underwater photograph of *S. Flava*, copies of the ¹H NMR, ¹³C NMR, 2D NMR, and ESI-MS spectra for isoneoamphilectane diterpenes **3–5**, coordinates and energies for all computed structures (Tables 4–6), and plausible biosynthetic pathways. This material is available free of charge via the Internet at <http://pubs.acs.org>.

isoneoamphilectane scaffolds are considered exceptionally rare, and to this date, isocyanides **1** and **2** remain as the first and only known representatives of these families of marine-derived diterpenes, respectively.^{9–11} Specifically, tricyclic compound **1** was isolated in 1992 by Higa and co-workers from a Japanese sponge of the family Adocidae,⁹ whereas **2**, formally a 2(1→12)-abeoamphilectane, was isolated in 1996 by König *et al.* from the tropical marine sponge *Cymbastela hooperi*.¹⁰ Often, amphilectane-based diterpenes known to contain isocyanide and related functionalities are endowed with potent *in vitro* anti-malarial, anti-algal, anti-tubercular, anti-bacterial, anti-photosynthetic, anti-inflammatory, anti-proliferative, and anti-fouling activity.^{8,10,12} Several investigations into the structure–activity relationships of these metabolites clearly suggest a connection between such nitrogen-containing functionalities and the observed patterns of pharmacological activity.¹²

As part of our ongoing quest for novel biologically active natural products from marine sponges of the Caribbean region, we report herein on the extraction, isolation, and structure elucidation of two new isoneoamphilectane-based diterpenes, 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**) and 7-formamidoisoneoamphilecta-1(14),15-diene (**4**), from the *n*-hexane extract of the Bahamian sponge *Svenzea flava* (Lehnert & van Soest, 1999) (Phylum Porifera; Class Demospongiae; Order Halichondrida; Family Dictyonellidae).^{13,14} Their structures, including relative configuration, were elucidated on the basis of 1D and 2D NMR in conjunction with IR, UV, and HRMS spectroscopic evidence. Furthermore, the absolute configuration of **2–4** was determined indirectly via density functional theory (DFT) and calculated vibrational circular dichroism (VCD) techniques.¹⁵ Briefly, we calculated the VCD and IR spectra for both enantiomers of semi-synthetic derivative **5**, which were subsequently compared to its experimental VCD spectrum thus establishing the absolute configuration about its stereogenic centers. All isolates, but especially analog **5**, demonstrated strong *in vitro* activity against the tuberculosis causative pathogen *Mycobacterium tuberculosis* H₃₇Rv.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Diterpenes **3** and **4**

A small specimen of *S. flava* (190 g of lyophilized sponge material) was extracted repeatedly with a 1:1 mixture of CHCl₃–MeOH and, after filtration and concentration of the combined extracts, the orange gum isolated was suspended in H₂O and partitioned against *n*-hexane. *In vitro* antituberculosis screening of the dry *n*-hexane solubles revealed significant inhibitory activity against *M. tuberculosis* H₃₇Rv (MIC = 15.1 µg/mL). Bioassay-guided fractionation of the extract using vacuum liquid chromatography (VLC) over silica gel in tandem with NMR (¹H/¹³C), IR, and TLC analyses of the active fractions, led subsequently to the isolation of two new metabolites, 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**)

and 7-formamidoisoneoamphilecta-1(14),15-diene (**4**), along with the following known compounds: 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**),¹⁰ (–)-8,15-diisocyano-11(20)-amphilectene (**6**),^{1a} 8-isocyano-11(20)-ene-15-amphilectaformamide (**7**),^{1a} and 7,15-diisocyano-11(20)-amphilectene (**8**).^{2,16} All of the known isolates were characterized unambiguously by spectroscopic analysis, including ESI-MS, UV, IR, $[\alpha]_D$, and NMR spectra, and by comparisons with data from previously published reports.

The ESI-MS (positive ions) of 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**), isolated as a colorless oil, showed a pseudomolecular ion peak at m/z 302 $[M+H]^+$. Accurate mass measurement of this peak assigned the molecular formula $C_{21}H_{35}N$ to **3**. The IR spectrum revealed bands that were consistent with the presence of N—H (3404 cm^{-1}) and alkene ($3078, 1639, 970$ and 881 cm^{-1}) functionalities. The presence of four peaks in the ^{13}C NMR spectrum ascribable to sp^2 -hybridized carbons at δ_C 142.5 (C, C-15), 138.0 (CH, C-1), 129.4 (CH, C-14), and 114.1 (CH_2 , C-16) established the presence of two carbon—carbon double bonds that had the aspects of being conjugated [UV(hexane), λ_{max} 234 nm (ϵ 16000)]. The 1H and ^{13}C NMR spectra of **3** ($CDCl_3$, Table 1) were analyzed with the help of a 2D NMR HSQC experiment, suggesting the presence of four methyls at δ_H 1.84 (s), 0.97 (s), 0.92 (d, $J = 6.4$ Hz), and 0.76 (d, $J = 6.4$ Hz), an additional methyl singlet at δ_H 2.33, six methylenes (one sp^2 and five sp^3), seven methines (two sp^2 and five sp^3), two quaternary carbons (one sp^2 and one sp^3), and one unprotonated sp^3 carbon atom bearing nitrogen. Thus, in order to account for the five unsaturation degrees implied by the molecular formula, **3** must be tricyclic. After all proton and carbon resonances had been associated from the results of 1H – ^{13}C shift correlated 2D NMR measurements (HSQC, $J = 150$ MHz), it was possible to deduce the planar structure of **3** from data subtracted from its 1H – 1H DQF-COSY and HMBC ($J = 6.0$ and 8.0 Hz) spectra. Critically, inspection of the 1H – 1H COSY spectrum allowed us to deduce the three spin systems evidenced with bold linkages in Figure 1, while analysis of the HMBC spectrum provided information to join the above moieties, thus building up the planar structure of compound **3** (Table 1). In particular, $^{2,3}J$ HMBC correlations of the methyl protons appended at C-3, C-7, and C-11 indicated the presence of a perhydroacenaphthene ring that, in turn, was connected to the C_5 acyclic tail thanks in no small part to the simultaneous correlations of C-12 (δ_C 46.1, C) with H-1, H-2 $\alpha\beta$, H-11, H-13, and H₃-20.¹⁷ After its 1H and ^{13}C NMR data had been recorded and assigned (Table 1), it dawned upon us that **3** was essentially identical to prototype **2** with the exception of the substituent at C-7. On the basis of IR, ESI-MS, and NMR (1H and ^{13}C) spectroscopic evidence, this substituent clearly had to be a methylamino function. The proposed structure elucidation of **3** was subsequently confirmed by analysis of the HSQC-TOCSY ^{13}C – 1H shift correlated spectrum to furnish its structure as a new isoneoamphilectane congener, namely, 7-methylaminoisoneoamphilecta-1(14),15-diene.

Stereochemically, **3** was proposed to be identical to **2** on the basis of the almost identical ^{13}C NMR data and optical rotation comparisons. Inasmuch as the optical rotations of **2** and **3** in $CHCl_3$ solution were comparable ($[\alpha]_D$ values: +57.0 and +27.0, respectively),¹⁸ as were the two sets of 1H and ^{13}C NMR data for comparable centers, it appeared most likely that these two molecules had identical constitution and equal absolute configuration. Indeed, the structural relationship of these compounds was decisively

demonstrated when isocyanide **2**, upon reduction with LiAlH_4 in THF at 25 °C, yielded **3** in 51% isolated yield.¹⁹ On the basis of these results, it was concluded that the new molecule is (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*S**,13*R**)-7-methylaminoisoneoamphilecta-1(14),15-diene.

The accurate molecular mass of 7-formamidoisoneoamphilecta-1(14),15-diene (**4**) indicated it to have the molecular formula $\text{C}_{21}\text{H}_{33}\text{NO}$. From its UV and 1D/2D NMR data (see Figure 1 and Table 2) it appeared that **4** also was an isoneoamphilectane derivative containing the same conjugated diene functionality as **2** and **3** [UV(MeOH), λ_{max} 232 nm (ϵ 9000)]. From the IR and NMR data of **4**, it was also evident that the only differences between **2** and **4** were a result of the C-7 isocyanide functionality in **2** emerging as a formamide in **4**. The presence of the latter functionality in **4** was further supported by the existence of two sets of NMR signals in a 2:1 ratio due to the two possible geometries a formamide adopts on the NMR time scale (*i.e.* **4a** and **4b**; Table 2).^{1,10} Close comparison of the NMR data for **4** with those for **2** and **3** led to the deduction that the formamide functionality resided at C-7 (δ_{C} 163.0, 57.0 [*trans*]; 160.4, 58.6 [*cis*]), showing **4** to be the 7-formamido derivative of 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**). Contrary to previous reports on the geometry of the formamide group, the coupling constants of 7-NHCHO (δ 8.26, d, J = 12.3 Hz for the major rotamer **4a**, and δ 8.04, d, J = 2.1 Hz for the minor **4b** rotamer) clearly indicated the *transoid* conformation to be the dominant species.^{3b,4} As the NMR data of **2–4** were virtually identical for comparable stereocenters, and the fact that the optical rotation of **4** ($[\alpha]_{\text{D}} +44.0$) was of similar magnitude and sign to those of **2** and **3**, it is likely that these three molecules all have identical absolute configuration. Furthermore, hydrolysis of isocyanide **2** with 98% AcOH yielded **4** in 65% yield demonstrating conclusively that the latter compound is in fact (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*S**,13*R**)-7-formamidoisoneoamphilecta-1(14),15-diene.

Absolute Configuration of the Tricyclic Istoneoamphilectane Core of Diterpenes 2–5

Oddly, the initial isolation of isocyanide **2** that led to the determination of its structure by extensive NMR spectroscopy studies and to the initial report of its biological activity, did not provide for the structural determination of its molecular stereochemistry.¹⁰ As the determination of the absolute configuration of chiral molecules is an important aspect of molecular stereochemistry, we decided to undertake this crucial task by using vibrational circular dichroism (VCD), an increasingly popular method for the unambiguous assignment of absolute configuration in chiral molecules.^{15,20} To achieve this worthwhile endeavor, we pursued the following strategy. While all of the isoneoamphilectanes in hand could serve as

bona fide candidates for VCD analysis, we chose to work with **4**, the most abundant analog. However, as the 7-formamide functionality undergoes *cis/trans* isomerization that would otherwise interfere with our investigation, **4** was transformed into 7-amino derivative **5**, which does not isomerize, preserves the original chirality, and requires the least amount of computational time. Thus, we begun our study by building compounds **5** and *ent*-**5** with HyperChem,²¹ and then conducted a conformational search for **5** at the molecular mechanics level.²² Then the geometry optimization, frequency, IR, and VCD intensity calculations of the conformers resulting from the conformational search were carried out at the DFT level [B3LYP functional/ 6–31G(d) basis set]²³ with Gaussian 09.²⁴ The calculated frequencies were scaled by 0.966 (the best scaling factor giving the best agreement between the experimental and calculated spectra)^{23,25} and the IR and VCD intensities were converted to Lorentzian bands with 6-cm⁻¹ half-width for subsequent comparisons to the experimental data.

The Gaussian calculations resulted in three conformers (Figure 2) that have energies within 0.4 kcal/mol from the lowest-energy conformer, and all the other conformers are more than 2 kcal/mol higher than the lowest-energy conformer. The optimized geometries, the relative energies and the Boltzmann populations of the three lowest-energy conformers for the 12*S* configuration are shown in Figure 2. Comparing the observed VCD and IR spectra with those of the calculated VCD of the conformers of 12*S* configuration (Figures 3 and 4A), it is clear that the chirality of C-12 is *S* based on the signs of the VCD bands. Therefore, the complete absolute configuration of **5** was determined as 3*S*, 4*R*, 7*S*, 8*S*, 11*R*, 12*S*, and 13*R*. The assignment was also evaluated by CompareVOA algorithm²⁵ and the confidence level of the C-12 assignment of *S* is 100% based on a current database that includes 105 previously correct assignments for different chiral structures (Figure 4B).

Bioactivity

Naturally-occurring isoneoamphilectanes **2–4**, as well as semisynthetic derivative **5**, were evaluated against the *Mycobacterium tuberculosis* strain H₃₇Rv. Albeit all of the isolates were active, surprisingly, semi-synthetic analog **5** displayed the most inhibitory activity with an MIC value of 6 µg/mL (Table 3). The realization that derivative **5** is so much more active than the natural products **3** and **4**, taken together with the fact that it showed no significant cytotoxicity against mammalian cells in the VERO cell assay (IC₅₀ > 128 µg/mL), is clearly of some significance. Conceivably, these data suggest that the isoneoamphilectane backbone is a key pharmacophoric moiety and might represent a novel scaffold for the development of clinically useful agents for tuberculosis. With respect to the toxicological effects of these analogs on mycobacteria, research is still necessary in order to understand the mechanism of action. Unfortunately, issues associated with the availability of these compounds have prevented us from further pursuing these investigations.

CONCLUSIONS

Arguably the most significant finding of this study was the discovery of the second and third examples of an exceptionally rare class of natural products, namely, the isoneoamphilectanes. For almost two decades, this family of metabolites has been

conformed by a single member. A comprehensive listing of fully reported and assigned ^1H – ^{13}C NMR data (Tables 1 and 2) for the new analogs **3** and **4** was provided, and these data should facilitate the assignment of structurally similar, yet to be discovered, natural products. Although each metabolite could be derived chemically from reduction or hydrolysis of isocyanide **2**, we did not address experimentally whether **3** and **4** are formed biosynthetically. Compounds **2**–**5** were tested for their *in vitro* biological activity against *M. tuberculosis* H₃₇Rv. The results of these assays (Table 3) showed only derivative **5** to have anti-tubercular activity that can be regarded as significant, and somewhat selective, based on its lack of cytotoxicity toward VERO cells. Furthermore, our data suggest that the isoneoamphilectane backbone, and not the isocyanide functionality, may well be the molecular feature responsible for the observed activity. For the first time, the absolute configuration of the isoneoamphilectane backbone has been designated as 3*S*, 4*R*, 7*S*, 8*S*, 11*R*, 12*S*, and 13*R*. The present work can serve as another demonstration for a successful application of vibrational circular dichroism spectroscopy, establishing it as an easy method to designate the absolute configuration of suitable molecules. Future synthetic work undertaken on the basis of these natural product leads would clearly demonstrate the potential of these diterpenes as important prototype structures for new anti-tubercular agents. With no structural ambiguities left, many groups might now embarked on the total synthesis of these structurally challenging natural products along with different stereoisomers as targets suited to pursue structure-activity studies.²⁶

EXPERIMENTAL SECTION

General Experimental Methods

Optical rotations were measured in CHCl_3 at 589 nm using a 10-cm microcell. IR spectra were recorded as neat films employing an FT-IR spectrometer with 4-cm^{-1} resolution. The UV spectra were recorded from 200 to 800 nm using a pathlength of 10 mm. Exact mass measurements were performed with a hybrid quadrupole time of flight mass spectrometer equipped with an electrospray (ESI) ion source. ESI samples were introduced through direct infusion into the ESI source using a syringe pump and MeOH as the solvent. For positive-ion mode, 0.1% formic acid or acetic acid was usually added into the analyte solution to enhance protonation and increase sensitivity. 1D and 2D NMR spectra were recorded at 500 MHz. ^1H and ^{13}C NMR chemical shifts are reported in ppm relative to the residual CHCl_3 signal (7.26 ppm) and CDCl_3 signal (77.0 ppm), respectively. Saturated solutions of anhydrous NH_3 in MeOH were prepared by bubbling dry gaseous NH_3 into dry MeOH. The percentage yield of each compound is based on the weight of the dry sponge specimen.

Animal Material

The Caribbean sponge *Svenzea flava* (Phylum Porifera; Class Demospongiae; Order Halichondrida; Family Dictyonellidae) was collected at a depth of 80–90 feet by scuba off Great Inagua Island, Bahamas, in July 2011. A voucher specimen (No. SV11-07) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The species *Svenzea flava* was originally classified as *Pseudoaxinella flava*.¹³ Despite lacking dark granulous cells that are a signature characteristic of other species within the genus *Svenzea*, it has been accepted as *Svenzea flava*.¹⁴

Extraction and Isolation

Shortly after collection, the frozen sponge specimen was lyophilized, the dry specimen (190 g) cut into small pieces, and blended with a 1:1 mixture of CHCl_3 –MeOH ($6 \times 1\text{L}$). The combined extract was filtered and concentrated *in vacuo* to yield an orange gum (13.2 g) that was later suspended in H_2O (2 L) and partitioned against solvents of increasing polarity (*n*-hexane, CHCl_3 , EtOAc) ($4 \times 500\text{ mL}$). After concentration of the combined *n*-hexane extracts, the orange oily residue (5.9 g) left over was subjected to vacuum liquid chromatography (VLC) over silica gel (150 g) using a *n*-hexane/EtOAc gradient (98:2–1:1) to give five fractions (denoted I–V) on the basis of *in-house* biological activity assays and routine TLC and ^1H NMR analyses. Further purification of fraction-II (1.2 g) by silica gel (20.0 g) column chromatography (CC) using 2% *n*-hexane/EtOAc afforded 15 sub-fractions, denoted as A–N. Sub-fraction C was identified as the known compound 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**) (12 mg, 0.006%) on the basis of IR, UV, $[\alpha]_D$, MS and ^1H - and ^{13}C NMR spectroscopic analyses.¹⁰ Sub-fraction E consisted of a pure colorless crystalline solid that was subsequently identified as known (–)-8,15-diisocyano-11(20)-amphilectene (**6**) (108 mg, 0.06%) after X-ray crystallographic analysis.^{1a} Similarly, subfractions F and J were subsequently identified as the known compounds ergosterol (24 mg, 0.01%)²⁷ and 7,15-diisocyano-11(20)-amphilectene (**8**) (37 mg, 0.02%),² respectively, by spectroscopic analysis and from comparisons to the known literature data. Analysis of the data obtained for sub-fraction H revealed that this portion consisted of new 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**) (7 mg, 0.004%). Purification of sub-fraction L by CC over a short plug of silica gel (1.0 g) using 2% MeOH– CHCl_3 yielded pure 7-formamidoisoneoamphilecta-1(14),15-diene (**4**) (14 mg, 0.007%). Lastly, sub-fraction M was recognized as the previously known 8-isocyano-11(20)-ene-15-amphilectaformamide (**7**) (46 mg, 0.02%).^{1a}

7-Methylaminoisoneoamphilecta-1(14),15-diene (3)—colorless oil; $[\alpha]_D^{20} +27.0$ (*c* 1.0, CHCl_3); UV (hexane) λ_{max} (log ϵ) 234 (4.2) nm; IR (film) ν_{max} 3404, 3078, 3022, 2949, 2925, 2856, 2796, 1639, 1606, 1475, 1456, 1375, 1170, 1109, 970, 881 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3) (see Table 1); HRMS (ESI) $[\text{M}+\text{H}]^+ m/z$ 302.2856 (calcd for $\text{C}_{21}\text{H}_{36}\text{N}$, 302.2848).

7-Formamidoisoneoamphilecta-1(14),15-diene (4)—colorless oil; $[\alpha]_D^{20} +44.0$ (*c* 1.0, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 232 (3.9) nm; IR (film) ν_{max} 3296, 3053, 2947, 2927, 2868, 1679, 1535, 1456, 1377, 970, 885, 756 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3) (see Table 2); HRMS (ESI) $[\text{M}+\text{H}]^+ m/z$ 316.2651 (calcd for $\text{C}_{21}\text{H}_{34}\text{NO}$, 316.2640).

Reduction of 7-Isocyanoisoneoamphilecta-1(14),15-diene (2) with LiAlH_4

Isocyanide **2** (2.3 mg, 0.008 mmol) in anhydrous THF (0.5 mL) was added slowly to a suspension of LiAlH_4 (1.2 mg, 0.03 mmol) in THF (0.5 mL) under argon. The reaction mixture was stirred for 20 h at 25 °C before it was quenched with 1 mL of MeOH. After filtering the resulting slurry through a short plug of Celite®, CHCl_3 (5 mL) was added and the solution washed with brine, dried, concentrated, and purified by CC on silica gel (1.0 g,

99:1 (v/v) MeOH in CHCl₃) to give 1.2 mg (51%) of **3**; all solution spectral and TLC data were identical with those obtained from authentic material.

Basic Hydrolysis of 7-Formamidoisoneoamphilecta-1(14),15-diene (**4**)

A mixture of formamide **4** (6 mg, 0.02 mmol) and KOH 1M (5 mL) in freshly distilled MeOH (10 mL) was slowly heated to 60 °C with constant stirring for 12 h. After cooling to rt, the reaction mixture was concentrated *in vacuo* and the oily residue obtained suspended in H₂O (5 mL) and extracted with CHCl₃ (3 × 5 mL). The combined organic layer was dried (MgSO₄), filtered, and concentrated to leave a residue that was purified over silica gel (300 mg) using a mixture of 2% MeOH(NH₃) in CHCl₃ to afford 7-aminoisoneoamphilecta-1(14),15-diene (**5**) (4.9 mg, 90% yield): colorless oil; [α]_D²⁰ +28.0 (*c* 1.0, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 232 (4.0) nm; IR (film) ν_{max} 3445, 3354, 2950, 2925, 2868, 1639, 1606, 1566, 1456, 1365, 970, 881 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 6.11 (d, 1H, *J* = 16.1 Hz, H-14), 5.67 (d, 1H, *J* = 16.1 Hz, H-1), 4.89 (br s, 2H, H-16), 2.12 (m, 1H, H-2), 1.84 (s, 3H, H-17), 1.79 (m, 1H, H-5), 1.70 (m, 1H, H-6), 1.69 (m, 1H, H-9), 1.60 (m, 1H, H-10), 1.58 (m, 1H, H-11), 1.42 (m, 1H, H-3), 1.31 (m, 1H, H-6'), 1.28 (m, 1H, H-8), 1.27 (m, 1H, H-10'), 1.23 (m, 1H, H-13), 1.14 (m, 1H, H-5'), 1.02 (m, 1H, H-2'), 1.01 (m, 1H, H-9'), 1.00 (s, 3H, H-19), 0.91 (d, 3H, *J* = 6.4 Hz, H-18), 0.90 (m, 1H, H-4), 0.76 (d, 3H, *J* = 6.4 Hz, H-20); ¹³C NMR (CDCl₃, 125 MHz) δ 142.5 (C, C-15), 137.9 (CH, C-1), 129.4 (CH, C-14), 114.1 (CH₂, C-16), 55.0 (CH, C-4), 54.9 (CH, C-13), 53.2 (C, C-7), 49.1 (CH, C-8), 49.0 (CH₂, C-2), 46.1 (C, C-12), 44.3 (CH₂, C-6), 37.3 (CH, C-3), 35.7 (CH, C-11), 28.6 (CH₂, C-10), 27.7 (CH₂, C-5), 21.6 (CH₃, C-19), 19.6 (CH₂, C-9), 18.9 (CH₃, C-17), 17.0 (CH₃, C-18), 15.9 (CH₃, C-20); HRMS (ESI) [M+H]⁺ *m/z* 288.2692 (calcd for C₂₀H₃₄N, 288.2691).

Acid Hydrolysis of 7-Isocyanoisoneoamphilecta-1(14),15-diene (**2**)

A homogeneous mixture of isocyanide **2** (10 mg, 0.031 mmol) and glacial AcOH (3 drops) dissolved in freshly distilled MeOH (10 mL) was stirred at 25 °C for 24 h. After concentration, the resulting solid residue left over was purified by flash CC over silica gel using 2% MeOH(NH₃)–CHCl₃ to afford 7.2 mg (65%) of 7-formamidoisoneoamphilecta-1(14),15-diene (**4**).

VCD Analysis and DFT Calculations

Semi-synthetic derivative 7-aminoisoneoamphilecta-1(14),15-diene (**5**) (3.8 mg) was dissolved in CDCl₃ (3.8 mg/0.15 mL) and placed in a 100 μ m path-length IR cell with BaF₂ windows. IR and VCD spectra were recorded on a ChiralIR2XTM VCD spectrometer (BioTools, Inc.) equipped with dual photo elastic modulator (PEM) accessory with 4 cm⁻¹ resolution, 20 h collection for both the sample and CDCl₃, and the two PEMs instruments optimized at 1400 cm⁻¹. Both the IR and the VCD baselines were obtained by subtracting the solvent spectra from the sample spectra. The geometry optimization, IR and VCD intensities of all the conformers generated by conformational search using HyperChem were calculated with a Linux AMD64 2.4 GHz using the Gaussian 09.²⁴ The comparison between the observed and the calculated spectra were evaluated by the CompareVOA program developed by BioTools.^{25,28}

Anti-tubercular Activity Bioassays

The anti-tubercular activity of isoneoamphilectane diterpenes **2–5** was evaluated against the laboratory strain *Mycobacterium tuberculosis* H₃₇Rv. A detailed description of the experimental methods used has been previously described.²⁹ All of the anti-tubercular assays were performed at the Institute for Tuberculosis Research, University of Illinois at Chicago, College of Pharmacy, 833 S. Wood Street (M/C 964) Chicago, IL by members of Professor Scott G. Franzblau's Research Group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Dr. Scott G. Franzblau and Dr. Yuehong Wang from the Institute for Tuberculosis Research (University of Illinois at Chicago) for the antituberculosis bioactivity data and Dr. Yanan He (BioTools, Inc., Jupiter, FL) for providing the calculated and experimental VCD spectra for compound **5**. Mass spectroscopic determinations were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana-Champaign. Financial support to E. Avilés was generously provided by the GK-12 Fellowship (Grant No. 0841338). We thank Dr. Joseph R. Pawlik (UNC-Wilmington) for graciously allowing one of us (J.V.) to participate in an expedition to the Bahamas, during which the sponge *S. flava* was collected. This research was supported by a grant from the NIH-SC1 Program (Grant 1SC1GM086271-01A1) awarded to A.D. Rodríguez.

References

1. (a) Wratten SJ, Faulkner DJ, Hirotsu K, Clardy J. Tetrahedron Lett. 1978;4345–4348.(b) Avilés E, Rodríguez AD. Org Lett. 2010; 12:5290–5293. [PubMed: 20964325]
2. (a) Ciavatta ML, Fontana A, Puliti R, Scognamiglio G, Cimino G. Tetrahedron. 1999; 55:12629–12636.(b) Ciavatta ML, Gavagnin M, Manzo E, Puliti R, Mattia CA, Mazzarella L, Cimino G, Simpson JS, Garson MJ. Tetrahedron. 2005; 61:8049–8053.
3. (a) Mitome H, Shirato N, Miyaoka H, Yamada Y, van Soest RWM. J Nat Prod. 2004; 67:833–837. [PubMed: 15165146] (b) Chanthathamrongsiri N, Yuenyongsawad S, Wattanapiromsakul C, Plubrukarn A. J Nat Prod. 2012; 75:789–792. [PubMed: 22376176] (c) Wattanapiromsakul C, Chanthathamrongsiri N, Bussarawit S, Yuenyongsawad S, Plubrukarn A, Suwanborirux K. Can J Chem. 2009; 87:612–618.
4. (a) Linden A, König GM, Wright AD. Acta Cryst. 1996; C52:2601–2607.(b) Wright AD, Lang-Unnasch N. J Nat Prod. 2009; 72:492–495. [PubMed: 19199790]
5. (a) Baker JT, Wells RJ, Oberhansli WE, Hawes GB. J Am Chem Soc. 1976; 98:4010–4012.(b) Kazlauskas R, Murphy PT, Wells RJ, Blount JF. Tetrahedron Lett. 1980; 21:315–318.
6. Molinski TF, Faulkner DJ, Van Duyne GD, Clardy J. J Org Chem. 1987; 52:3334–3337.
7. Ciasullo L, Cutignano A, Casapullo A, Puliti R, Mattia CA, Debitus C, Riccio R, Gomez-Paloma L. J Nat Prod. 2002; 65:1210–1212. [PubMed: 12193036]
8. Lamoral-Theys D, Fattorusso E, Mangoni A, Perinu C, Kiss R, Costantino V. J Nat Prod. 2011; 74:2299–2303. [PubMed: 21985105]
9. Sharma HA, Tanaka J, Higa T, Lithgow A, Bernardinelli G, Jefford CW. Tetrahedron Lett. 1992; 33:1593–1596.
10. König GM, Wright AD, Angerhofer CK. J Org Chem. 1996; 61:3259–3267.
11. Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. Nat Prod Rep. 2013; 30:237–323. and previous articles in this series. [PubMed: 23263727]
12. (a) Wright AD, Wang H, Gurrath M, König GM, Kocak G, Neumann G, Loria P, Foley M, Tilley L. J Med Chem. 2001; 44:873–885. [PubMed: 11300869] (b) Gross H, König GM. Phytochem Rev. 2006; 5:115–141.(c) Wright AD, McCluskey A, Robertson MJ, MacGregor KA, Gordon CP,

- Guenther J. *Org Biomol Chem*. 2011; 9:400–407. [PubMed: 21042642] (d) Mayer AMS, Avilés E, Rodríguez AD. *Bioorg Med Chem*. 2012; 20:279–282. [PubMed: 22153874]
13. Lehnert H, Van Soest RWM. *Beaufortia*. 1999; 49:141–169.
14. Alvarez B, Van Soest RWM, Rützler K. *Contrib Zool*. 2002; 71:171–176.
15. (a) He Y, Wang B, Dukor RK, Nafie LA. *Appl Spectrosc*. 2011; 65:699–722. [PubMed: 21740631] (b) Felipe LG, Batista JM, Baldoqui DC, Nascimento IR, Kato MJ, He Y, Nafie LA, Furlan M. *Org Biomol Chem*. 2012; 10:4208–4214. [PubMed: 22543980]
16. A small amount of the well-known sterol ergosterol was also isolated.
17. Rodríguez AD, Rodríguez II. *Tetrahedron Lett*. 2002; 43:5601–5604.
18. Our sample of 7-isocyanoisoneoamphilecta-1(14),15-diene (2) had $[\alpha]_D^{20} +57.0$ (c 1.0, CHCl₃). In the original study (ref. 10) 2 had $[\alpha]_D^{25} +67.0$ (c 0.79, CHCl₃).
19. Van Leusen D, Van Leusen AM. *Org React*. 2001; 57:417–679.
20. While X-ray crystallography is a straightforward and highly reliable method for establishing absolute configuration, this method requires a single crystal of the sample of sufficient size and quality. Moreover, an atom heavier than oxygen must generally be present in the molecule (or must be incorporated into the molecule by derivatization) in order to determine the absolute configuration of the crystal unambiguously (for an excellent review of the use and limitations of X-ray crystallography to determine absolute configuration, see: Flack HD, Bernardinelli G. *Chirality*. 2008; 20:681–690. Because none of the present isoneoamphilectanes met these requirements, we chose VCD. This method is the extension of electronic CD into the infrared region, where fundamental vibrational transitions occur. VCD has a number of advantages over the other methods of absolute configuration assignment, but has some limitations as well. Like X-ray crystallography, VCD is an absolute, not a relative method, but unlike X-ray analysis does not require the presence of a heavy atom in the molecule and is not restricted to single crystals. VCD spectra are recorded for neat liquid or solution in suitable solvents (CS₂, CCl₄, CDCl₃) and no derivatization of the molecule is required prior to analysis. [PubMed: 17924422]
21. Hypercube, Inc., 1115 NW 4th St, Gainesville, FL 32601.
22. We used the MMFF94 molecular mechanics force-field for the conformational search.
23. (a) Becke AD. *J Chem Phys*. 1993; 98:1372–1377. (b) Kim K, Jordan KD. *J Phys Chem*. 1994; 98:10089–10094. (c) Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ. *J Phys Chem*. 1994; 98:11623–11627.
24. Frisch, MJ.; Trucks, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, GA.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, HP.; Izmaylov, AF.; Bloino, J.; Zheng, G.; Sonnenberg, JL.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, JA., Jr; Peralta, JE.; Ogliaro, F.; Bearpark, M.; Heyd, JJ.; Brothers, E.; Kudin, KN.; Staroverov, VN.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, JC.; Iyengar, SS.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, JM.; Klene, M.; Knox, JE.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Martin, RL.; Morokuma, K.; Zakrzewski, VG.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Dapprich, S.; Daniels, AD.; Farkas, Ö.; Foresman, JB.; Ortiz, JV.; Cioslowski, J.; Fox, DJ. *Gaussian 09, Revision A.1*. Gaussian, Inc; Wallingford CT: 2009.
25. Debie E, De Gussem E, Dukor RK, Herrebout W, Nafie LA, Bultinck P. *Chem Phys Chem*. 2011; 12:1542–1549. [PubMed: 21542094]
26. For an insightful application of intramolecular Diels–Alder cycloadditions of fulvenes to a variety of polycyclic rings, including the isoneoamphilectane skeleton, see: Hong BC, Chen FL, Chen SH, Liao JH, Lee GH. *Org Lett*. 2005; 7:557–560. [PubMed: 15704893]
27. (a) Chobot V, Opletal L, Jahodar L, Patel AV, Dacke CG, Blunden G. *Phytochemistry*. 1997; 45:1669–1671. (b) Lee DI, Choi JS, Yang MR, Lee WK, Kim DS, Choi HD, Son BW. *Nat Prod Sci*. 1999; 5:93–96.
28. BioTools, Inc., 17546 Beeline Highway, Jupiter, Florida 33458.
29. Collins LA, Franzblau SG. *Antimicrob Agents Chemother*. 1997; 41:1004–1009. [PubMed: 9145860]

Figure 1.

Spin systems deduced through the ^1H - ^1H DQF-COSY spectra and key 2,3J H $\rightarrow$ C correlations exhibited by the HMBC spectra of compounds **3** and **4**.

Figure 2. Optimized geometries, relative energies, and Boltzmann populations of the three calculated lowest-energy 12*S* conformers of 7-aminoisoneoamphilecta-1(14),15-diene (**5**).

Figure 3. IR (lower frame) and VCD (upper frame) spectra observed for **5** (right axes) compared with calculated Boltzmann-population-weighted spectra of the three calculated lowest-energy conformers of the 12*S* configuration, (left axes).

Figure 4.

A. IR (lower frame) and VCD (upper frame) spectra observed for **5** (right axes) compared with the Boltzmann-averaged calculated spectra of the three lowest-energy conformations of the 12*S* configuration (left axes). **B.** CompareVOA program showing experimental VCD and IR spectra of compound **5** compared to the theoretical calculation of both enantiomers.

Scheme 1. Carbon Backbones for a Series of Spongian Amphilectane-Class Diterpenes and Their Trivial/Semisystematic Names^a

^a The names shown are consistent with the reports of Wright and Lang-Unnasch^{4b} and König *et al.*¹⁰ The numbers inside parentheses refer to the current number of sponge-derived analogs isolated per class of compound.

¹H NMR (500 MHz), ¹³C NMR (125 MHz), ¹H-¹H COSY, NOESY, and HMBC Spectroscopic Data for 7-Methylaminoisoneoamphilecta-1(14),15-diene (3)^a

position	δ _H , mult (J in Hz)	δ _C , type ^b	COSY	NOESY	HMBC ^c
1	5.68, d (16.1)	138.0, CH	H14	H2β, H10β, H17, H19, H20	H2αβ, H11, H13, H14
2α	1.00, br m	49.0, CH ₂	H2β, H3	H2β	H1, H3, H11, H13, H18
2β	2.12, dd (12.7, 5.5)		H2α, H3	H1, H2α, H3, H14	
3	1.42, br m	37.3, CH	Hαβ, H18, H4	H2β, H14	H2αβ, H4, H5αβ, H13, H18
4	0.85, br m	54.9, CH	H3, H5α, H13	H5α, H8	H2, H3, H5αβ, H6αβ, H8, H13, H18
5α	1.80, br m	27.3, CH ₂		H4, H6α, H18, H21	H3, H4, H6αβ
5β	1.11, br m		H4, H6α	H5α	
6α	1.41, br m	37.9, CH ₂		H5α, H6β	H4, H5αβ, H8, H19
6β	1.61, br m		H5β	H6α, H19	
7		56.1, C			H5αβ, H6αβ, H8, H9, H13, H19, H21
8	1.46, br m	44.7, CH	H9β	H4, H21	H4, H6αβ, H9αβ, H10αβ, H13
9α	0.96, br m	19.4, CH ₂		H9β, H10α	H8, H11, H10αβ, H13
9β	1.58, br m		H8, H10β	H9α, H10β, H19	
10α	1.58, br m	28.5, CH ₂		H9α, H10β	H8, H9αβ, H11, H20
10β	1.25, br m		H9β, H11	H1, H9β, H10α, H20	
11	1.57, br m	35.7, CH	H10β, H20		H1, H2αβ, H9αβ, H10αβ, H13, H20
12		46.1, C			H1, H2αβ, H4, H8, H10αβ, H11, H13, H14, H20
13	1.31, br m	54.2, CH	H4	H14, H19	H1, H2αβ, H3, H4, H5αβ, H8, H9αβ, H11
14	6.11, d (16.1)	129.4, CH	H1	H2α, H3, H13	H1, H16, H17
15		142.5, C			H1, H14, H16, H17
16	4.89, s	114.1, CH ₂			H14, H17
17	1.84, s	18.9, CH ₃		H1	H14, H16
18	0.92, d (6.4)	17.0, CH ₃	H3	H5α	H2αβ, H3, H4
19	0.97, s	19.0, CH ₃		H1, H6β, H9β, H13, H21	H6αβ, H8
20	0.76, d (6.4)	15.9, CH ₃	H11	H1, H10β	H11, H10αβ
21	2.33, s	28.0, CH ₃		H5α, H8, H19	

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

¹H NMR spectra were recorded in CDCl₃ at 25 °C. Chemical shift values are in ppm relative to the residual CHCl₃ (7.26 ppm) or CDCl₃ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, number of attached protons, and chemical shift values.

¹³C NMR types were obtained from a DEPTQ experiment.

Protons correlated to carbon resonances in the ¹³C column. Parameters were optimized for ^{2,3}J_{CH} = 6 and 8 Hz.

Table 2¹H (500 MHz) and ¹³C NMR (125 MHz) Spectroscopic Data for 4a and 4b^a

<i>Trans</i> rotational isomer 4a (major)			<i>Cis</i> rotational isomer 4b (minor)	
position	δ_{H} , mult (<i>J</i> in Hz)	δ_{C} , type ^b	δ_{H} , mult (<i>J</i> in Hz)	δ_{C} , type ^b
1	5.64, d (16.1)	137.1, CH	5.65, d (16.1)	137.6, CH
2 α	1.02, br m	48.8, CH ₂	1.02, br m	48.9, CH ₂
2 β	2.11, br m		2.11, br m	
3	1.42, br m	37.2, CH	1.42, br m	37.3, CH
4	0.92, br m	54.4, CH	0.98, br m	54.3, CH
5 α	1.82, br m	26.9, CH ₂	1.82, br m	27.1, CH ₂
5 β	1.19, br m		1.19, br m	
6 α	1.52, br m	43.0, CH ₂	1.99, br m	38.6, CH ₂
6 β	1.90, br m		2.12, br m	
7		57.0, C		58.6, C
8	1.47, br m	47.7, CH	2.07, br m	44.1, CH
9 α	1.06, br m	19.5, CH ₂	0.86, br m	19.9, CH ₂
9 β	1.59, br m		1.52, br m	
10 α	1.59, br m	28.1, CH ₂	1.59, br m	28.3, CH ₂
10 β	1.26, br m		1.26, br m	
11	1.56, br m	35.6, CH	1.56, br m	35.6, CH
12		46.0, C		46.1, C
13	1.27, br m	53.8, CH	1.27, br m	53.6, CH
14	6.10, d (16.1)	129.8, CH	6.10, d (16.1)	129.6, CH
15		142.2, C		142.4, C
16	4.88, br s	114.6, CH ₂	4.88, br s	114.4, CH ₂
17	1.82, s	18.8, CH ₃	1.82, s	18.9, CH ₃
18	0.91, d (6.2)	16.8, CH ₃	0.90, d (6.2)	16.9, CH ₃
19	1.24, s	19.3, CH ₃	1.25, s	18.9, CH ₃
20	0.76, d (6.2)	15.7, CH ₃	0.75, d (6.2)	15.8, CH ₃
21	8.26, d (12.3)	163.0, CH	8.04, d (2.11)	160.4, CH

^aSpectra were recorded in CDCl₃ at 25 °C. Chemical shift values are in ppm relative to the residual CHCl₃ (7.26 ppm) or CDCl₃ (77.0 ppm) signals.

^b¹³C NMR types were obtained from a DEPTQ experiment.

Table 3

In Vitro Anti-TB Activities of Isonoeamphilectane Diterpenes 2–5 against *M. tuberculosis* H₃₇Rv and Determination of Their Cytotoxicity Against the VERO Cell Line^a

Compound	% Inhibition in Test Conc. (128–8 µg/mL)					MIC ^b	Cytotoxicity ^c	
	128	64	32	16	8		<i>Mtb</i> H ₃₇ Rv strain	VERO cells
2	100	99	99	98	89	8	8	19
3	99	100	100	95	7	15	15	N.T.
4	99	100	91	30	17	32	32	N.T.
5	100	100	100	100	99	6	6	>128
RMP^d	100	100	100	100	99	0.05	0.05	89

^aValues are means of three experiments.

^bMinimum inhibitory concentrations (MIC) in µg/mL.

^cIC₅₀ in µg/mL. The cytotoxicity data for **2** (taken from ref. 10) are against mammalian KB cells. N.T. = Not tested.

^dRifampin (RMP) was used as a positive control.